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Summary

Summary: Hematopoietic antigen presenting cells in the human thymic cortex: Evidence for a role in selection and removal of apoptotic thymocytes.

The immune system has evolved to protect vertebrates against pathogenic micro-organisms, such as viruses, bacteria and parasites. T-cells, which originate from precursor cells residing in the bone marrow, are required in order to mediate a specific immune response against pathogens. Each naïve T-cell expresses a unique T-cell receptor (TCR), which is a transmembrane protein that enables recognition of foreign antigen when presented as peptides (small pieces of protein) in the groove of molecules encoded by the major histocompatibility complex (MHC). MHC molecules are expressed on the surface of antigen presenting cells (APC) that screen the body for the presence of invading pathogens. Upon encounter of a pathogen APCs take them up and process their proteins into peptides that can then be presented on MHC to T-cells. Only those T-cells that bear a TCR specific for such a pathogen-derived peptide in the context of MHC can mature and initiate an immune response against the pathogens.

In order to avoid an immune reaction to peptides derived from self-proteins a functional immune system requires the presence of T-cells expressing TCRs that are major histocompatibility complex (MHC) restricted but tolerant to self-antigens. This is achieved during selection T-cells in the thymus. T-cell precursors (thymocytes) enter the thymus where they develop into mature T-cells. Two selective events based on TCR recognition of peptide-MHC occur in the thymus, namely positive and negative selection. Positive selection results in the survival of only those thymocytes whose TCR interacts with self-MHC since T-cells that cannot recognize the individual's own MHC molecules could not mount an immune response against any antigen presented in the periphery. Thus positive selection results in a repertoire of T-cells that are capable to respond to foreign antigenic peptides when bound on self-MHC.

Furthermore, thymocytes that bind too strongly to self antigens are deleted during negative selection. Negative selection is required to ensure that no self-reactive T-cells can mature and leave the thymus into the peripheral circulation therewith avoiding self-attack or autoimmunity. Thus, positive and negative selection establishes a diverse T-cell repertoire with the capacity to react to foreign antigen but being tolerant self antigen.

The thymus consists of numerous lobules that are clearly differentiated into a cortical-outer and medullary-inner region. A network of thymic epithelial cells, also designated thymic stroma, provides a unique microenvironment necessary for the development of T-cells. Thymocytes in all stages of T-cell development can be found embedded in the stroma of the thymus.

Positive selection of thymocytes requires antigen presentation by thymic epithelial cells, specialized stromal cells located in the cortex of the thymus that express MHC class I and II complexes. Although this paradigm is widely accepted some studies have shown that in addition to cortical epithelial cells, bone marrow derived antigen presenting cells such as dendritic cells or macrophages participate at least to some extent in positive selection as well. Unfortunately, however, these cells have not been clearly identified and characterized to date in the human thymic cortex.

Negative selection occurs in the medulla of the thymus on bone marrow derived antigen presenting cells such as dendritic cells and macrophages. It is important to note that for negative selection a co-stimulatory signal in addition to the TCR-MHC interaction is required. For example the interaction of CD28 on thymocytes with CD80 or CD86 can provide this co-stimulatory signal.

Only a small population of thymocytes generated in the bone marrow or thymus survives T-cell selection and leaves the thymus as mature T-cells. The vast cell death occurring in the thymus is a reflection of the intensive screening that each thymocyte has to undergo during thymocyte selection to recognize self-MHC in a way that self tolerance is maintained. Most thymocytes undergo cell death because they fail to be positively selected and undergo “death by neglect” whereas negative selection makes only a small contribution to the background rate of apoptosis. In the murine thymus it has been shown that a specific population of cortical macrophages quickly removes these dying thymocytes from the cortex. In the human thymus the identity of the cell type responsible for the removal of the enormous numbers of dying cells in the thymic cortex has not been identified to date although it is tempting to assume that a similar cell type as in mice is responsible for this.

In this thesis a specific type of hematopoietic antigen presenting cells is identified in the cortex of the human thymus by expression of the C-type lectin DC-SIGN, which is a marker for human immature dendritic cells. These cortical cells exhibit features of both macrophages and dendritic cells. It is demonstrated that these DC-SIGN⁺ cells function in removal of apoptotic thymocytes from the cortex of the human thymus (Chapter 2). Furthermore co-expression of the molecular chaperone HLA-DM that is a hallmark of antigen presentation suggests an additional role of these cortical hAPC in antigen presentation and therefore T-cells selection. The cells are therefore termed cortical hematopoietic antigen presenting cells (hAPC).

Cortical hAPC are shown to prominently express the co-stimulatory molecule VCAM-1 (Chapter 3), which further underscores a potential function in antigen presentation whereas VCAM-1 is

not expressed in the medulla. VCAM-1 has been demonstrated to induce co-stimulation of peripheral T-cells. In this thesis it is shown that recombinant VCAM-1 also binds viable cortical thymocytes and moreover co-stimulates positive selection of thymocytes in combination with a TCR signal *in vitro*. Either a TCR or co-stimulatory signal alone does not result in positive selection of thymocytes. This suggests that co-stimulatory signals are not only required for negative selection of thymocytes but also for positive selection. VCAM-1 is most prominently expressed on cortical hAPC whereas the cortical epithelium does not or only weakly express VCAM-1. This highlights the potential importance of these cortical hAPC in positive selection of thymocytes.

Furthermore it is shown that the C-type lectin receptor DC-SIGN present on cortical hAPC specifically recognizes cortical apoptotic thymocytes, which is in line with the function of cortical DC-SIGN⁺ hAPC in removal of cortical thymocytes. Together these results suggest a dual function of cortical hAPC both in removal of apoptotic thymocytes as well as in positive selection of thymocytes.

Similar to DC-SIGN⁺ hAPC in the human thymic cortex F4/80⁺ macrophages present in the murine thymic cortex have been shown to clear apoptotic thymocytes from the cortex. In line with the results obtained in the human thymus these F4/80⁺ macrophages express the co-stimulatory molecule VCAM-1. Together this indicates that these cells are homologous and therefore implies a potential function of F4/80⁺ macrophages as antigen presenting cells in thymocyte positive selection in mice as well. In addition to VCAM-1 it is known that another co-stimulatory molecule namely ICAM-1 is expressed in the thymus. The co-stimulatory capacity of both VCAM-1 and ICAM-1 to induce positive selection is compared on murine thymocytes (Chapter 4). Both VCAM-1 and ICAM-1 show a similar capacity to co-stimulate positive selection of thymocytes, which is accompanied by survival of the selected cells.

Hence, co-stimulation appears to be necessary to induce positive selection. The expression of co-stimulatory molecules on cortical hAPC strongly suggests that besides cortical epithelial cells also the identified cortical hAPC play a role in positive selection.

The presence of the C-type lectin DC-SIGN in the thymus and the observation that this C-type lectin receptor plays a role in removal of apoptotic thymocytes resulted in the idea to study the function of other C-type lectins in the human thymus. Therefore the C-type lectin MGL was investigated in the thymus (Chapter 5). It is reported that MGL is strongly expressed by macrophages that are localized in close proximity to blood and lymph vessels present in septal regions of the thymus. MGL can interact with both blood and lymph vessels in a carbohydrate dependent manner. Transmigration studies of MGL⁺ macrophages through a layer of endothelial

cells reveal that MGL slows down migration through the endothelial cells. As in particular large aggregations of MGL⁺ macrophages were found laying in close proximity to blood vessels it is proposed that MGL may function to retain macrophages adjacent to vessels forming an arrangement that resembles splenic sinusoids. This is a structure that has been proposed to filter antigen from the blood stream. A similar function is proposed for MGL⁺ macrophages in the thymus.

The presence of DC-SIGN, MGL and other C-type lectin receptors in the human thymus specifically recognizing distinct carbohydrate moieties emphasize the importance of the specific recognition of glycoconjugates in the thymus. This initiated studies to obtain a better overview of glycosylation in the human thymus. To outline the distribution of glycoconjugates in microenvironments of the human thymus the glycosylation of the human thymic microarchitecture is studied by using plant lectins with defined carbohydrate specificity (Chapter 6). Thymic microenvironments such as cortex, medulla, interlobular space as well as lymph and blood vessels are shown to be marked by distinct glycosylation, which affirms the overall importance of glycosylation during various stages of T-cell development.

In conclusion, this work has identified novel cellular and molecular players in the human thymus involved in binding, selection and removal of thymocytes. In addition, the provided data points to an important role of glycosylation of thymic microenvironments that can be specifically recognized by C-type lectin receptors identified on various cell types in the thymus. Further in depth investigation of these novel players in the thymus will help to contribute to a better understanding of the mechanisms that govern T-cell development in the thymus.